

Tensile Properties of Hydrogels and of Snake Skin

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Introduction

Stimulus-responsive or “smart” gels are of potential interest as sensors and actuators, in industrial separations, and as permeable delivery systems [1]. In most applications, a certain degree of mechanical strength and toughness will be required, yet the large-strain behavior of gels has not been widely reported. Some exceptions include work on gelatin [2] and other food gels [3], some characterization of soft gels applicable for in-vitro cell growth studies [4], and toughness determinations on commercial contact lens materials [5]. In general, it can be anticipated that the gel stiffness will increase with increasing degree of crosslinking, but the tensile strength may go through a maximum [6].

Gel properties can be tailored by varying not only the degree of crosslinking, but also the polymer concentration and the nature of the polymer backbone (e.g. its stiffness or solubility). Polypeptides provide an especially interesting case, where secondary structure affects trends in moduli [7] and conformational transitions may accompany phase changes [8]. A few papers on the tensile properties of responsive gels have begun to appear [9, 10]. The responsive hydrogel chosen for the present study, crosslinked hydroxypropylcellulose, shrinks over a rather narrow temperature range near 44°C.

Some vertebrate skin is also subject to substantial strain. Among reptiles, the morphologies of the skin and scales show wide variations. Bauer *et al.* described the mechanical properties and histology of gecko skin [11]; longitudinal tensile properties of snake skin were examined by Jayne [12] with reference to locomotion. The present measurements focus on adaptations related to feeding, including the response of the skin to circumferential tension. Tensile properties will be related to interspecific and regional variation in skin structure and folding.

Experimental

Poly hydroxypropylcellulose, HPC, (Aldrich, mol wt= 10^5) was dissolved in deionized water and stirred gently overnight. Gels (10% and 20%) will be identified by the concentration of this solution. Divinyl sulfone (97%, Aldrich) crosslinker was added (0.18 g/g polymer) and the solution was thoroughly stirred by hand before NaOH (1M) was added to bring the solution to ca. pH 12 [13]. Finally, the solution was centrifuged briefly to remove air bubbles and poured into silicone molds capped with glass plates. The reaction was allowed to proceed at room temperature for 18-24 hours before immersing the specimens in dilute HCl and storing them in deionized water. Degree of swelling was determined by blotting and weighing samples equilibrated at the test temperatures and drying to constant weight.

Tensile specimens were scaled-down versions of a tapered geometry routinely used for rubber testing (ASTM D638, type V); gage sections were 0.8 x 1.5 x 18mm. Each specimen was affixed to a polyester film frame which served as a support and as gripping tabs. Testing was conducted at 1.3 cm/min crosshead speed in a misting chamber designed to expose the specimen to a continuous spray of deionized water; a thermistor probe verified the temperature of the spray. Strain was not measured directly, but approximate values were calculated from the crosshead displacement and the length of the straight section of the specimen.

To prepare the snake skin, adult male *thamnophis sirtalis* (14 individuals) were euthanized with sodium pentobarbital and circumferential strips of skin were excised at 10% increments of the snout-vent length or SVL. Thus the first strip was removed at 10% of SVL and the final one at 90% of SVL. Each of the nine strips (lettered a-i) spanned 10 ventral scales. Skin samples were stored on ice in reptilian Ringer's [14] and tested within 12 hours. The fresh skin, which had been split along the ventral midline, was gripped with aluminum clamps and tested at 10 mm/min crosshead speed. Results are reported as force per unit width of the skin sample. Samples for light microscopy were pinned to a vinyl pad in the relaxed or stretched states and preserved in 10% phosphate-buffered formalin; microtomed sections were stained with iron gallein [15] or Crowder's trichrome stain [16].

Results and discussion

Hydrogels. Mean tensile properties for the HPC gels both above and below the transition temperature are summarized in Table 1. Uncertainties quoted are those due to experimental scatter among 5 replicates.

Looking first at the room temperature data, we see that the sample with the higher polymer content is both stiffer and stronger than the more dilute network. The primary effect is not one of dilution, however, but of crosslink density. If classical Flory-Rehner theory [17] applies, the average molecular weights of network chains, M_c , can be estimated. The equilibrium swelling, combined with polymer-solvent interaction parameters quoted in reference [18], yields $M_c=51$ and 11 kg/mol, for the 10% and 20% gels, respectively, showing that the crosslinking reaction was much more efficient at the higher synthesis concentration. In spite of its higher strength, the 20% composition was rather difficult to handle because of its low elongation at break.

On going from room temperature to the 55°C (shrunk) state the modulus decreased for both compositions. This is somewhat unexpected; Takigawa *et al.*, for example, showed that a poly(N-isopropyl acrylamide) gel was 18-fold stiffer in the high-temperature, shrunk state [19]. They attributed the change to the formation of physical crosslinks (in addition to the chemical crosslinks introduced during the synthesis).

The larger elongation at break at 55° is characteristic [20] of what are called “supercoiled” networks (i.e. those produced by drying networks that were crosslinked in the swollen state). In the case of the 10% gel, this greater ductility, along with the smaller initial cross-sectional area due to shrinkage, translates to a substantially larger tensile strength at the higher temperature.

Table 1. Engineering tensile properties (average and standard deviation)

Test temperature, °C	Nominal (synthesis) concentration	Concentration at test temperature	Initial (tangent) modulus, kPa	Elongation at break, percent	Tensile strength, kPa
22	10%	10%	67±6	51±17	58±33
22	20%	20%	765±50	15±1	130±15
55	10%	60%	56±15	288±40	228±105
55	20%	54%	66±42	78±13	87±17

Even more interesting than the modulus and strength numbers, however, is the qualitative change in the shape of the load/deflection curves on going from 22° to 55°C. Both concentrations show inflections, and at 55°C the 10% gel especially is extremely soft during the early part of the curve (Figure 1). The plateau in the load (seen most clearly in the 10% network) is suggestive of coupling between the stretching and a phase change. For example, a collapsed polymer chain may exhibit a coil-globule transition [21, 22]. Stretching of such a chain may occur not by uniform elongation, but by chain segments “reeling out” [23] from the globule.

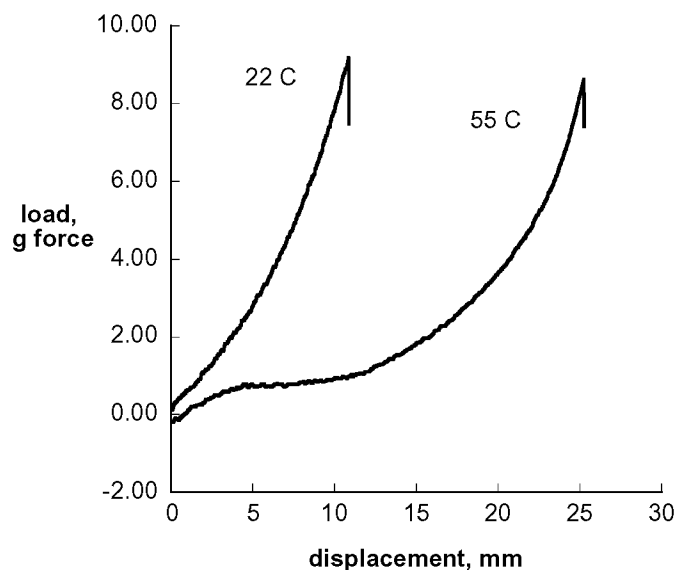


Figure 1. Load-displacement records for 10% gel at two temperatures.

It is not known whether HPC chains collapse in this fashion. It is known, however, that they can form cholesteric mesophases. HPC gels in an organic solvent [24] exhibited a plateau in the stress-strain behavior as had been predicted for nematic liquid crystal elastomers [25]. The

isotropic-to-biphasic critical concentration [26] for HPC in water is ca. 39% at room temperature, so our shrunken gels could plausibly be liquid-crystalline.

Snake skin. When stretched circumferentially, the snake skin initially provided very little resistance. This can be understood by examining cross-sectional micrographs (prepared in collaboration with Victor R. Townsend, Jr.) which show how folds of skin are “stored” under the edges of the scales. During the initial stage of stretching, these folds straighten with minimal stress. Elastin fibers in the stratum compactum and in a layer underlying the dermis may provide elasticity to refold the skin.

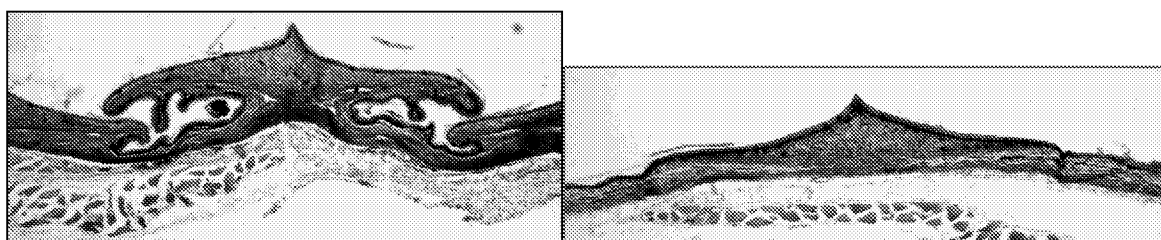


Figure 2. Micrographs of sections through skin of *Nerodia fasciata* (broad-banded water snake). Trichrome stain. Ridge at center of each photo runs along the long dimension of the scale (parallel to body). Left: unstretched. Right: stretched

Beyond the low-stress region, stress rises approximately linearly with elongation. By several measures, the skin near the head is much softer, as would be expected for an animal that needs to swallow large prey (Figure 3).

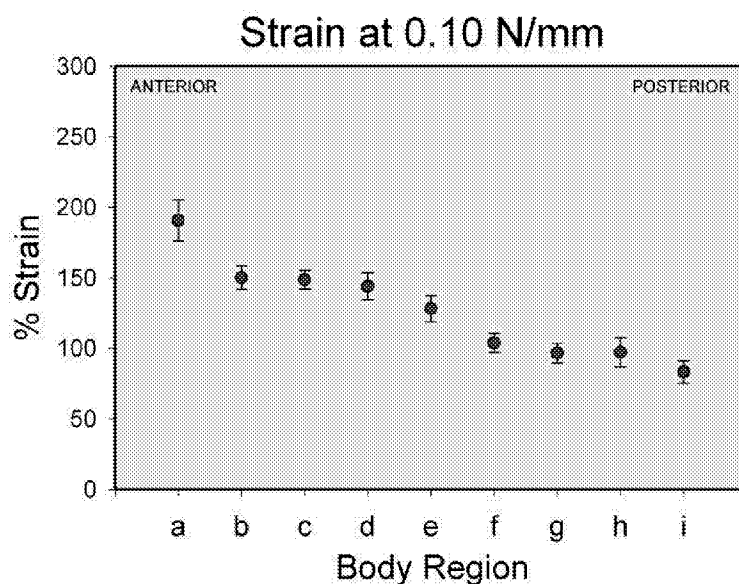


Figure 3. Strains at which stress reached 0.10 N/mm (read from load – elongation curves). Error bars represent 1 S.E.

Conclusions

Procedures have been developed to test small samples of temperature-responsive hydrogels. Stiffnesses, strengths, and failure strains in shrunken HPC gels cannot be predicted from data on the swollen state. A change in the molecular deformation mechanism is implied.

The skin of advanced snakes accommodates stretching through the arrangement of its fibrous constituents; regional variations in the morphology and mechanical properties of the skin are consistent the demands of feeding on large prey. Together these studies emphasize the morphological and mechanical complexity of hydrogels and highly extensible biological tissues.

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